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ORIGINAL ARTICLE

The effectiveness of demineralized cortical bone matrix in a chronic rotator cuff tear model

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Background: The purpose of this study was to assess the effect of demineralized bone matrix (DBM) on rotator cuff tendon–bone healing. The hypothesis was that compared with a commercially available dermal matrix scaffold, DBM would result in a higher bone mineral density and regenerate a morphologically superior enthesis in a rat model of chronic rotator cuff degeneration.

Methods: Eighteen female Wistar rats underwent unilateral detachment of the supraspinatus tendon. Three weeks later, tendon repair was carried out in animals randomized into 3 groups: group 1 animals were repaired with DBM (n = 6); group 2 received augmentation with the dermal scaffold (n = 6); and group 3 (controls) underwent nonaugmented tendon-bone repair (n = 6). Specimens were retrieved at 6 weeks post-operatively for histologic analysis and evaluation of bone mineral density.

Results: No failures of tendon-bone healing were noted throughout the study. All groups demonstrated closure of the tendon-bone gap with a fibrocartilaginous interface. Dermal collagen specimens exhibited a disorganized structure with significantly more abnormal collagen fiber arrangement and cellularity than in the DBM-based repairs. Nonaugmented repairs exhibited a significantly higher bone mineral density than in DBM and the dermal collagen specimens and were not significantly different from control limbs that were not operated on.

Conclusion: The application of DBM to a rat model of chronic rotator cuff degeneration did not improve the composition of the healing enthesis compared with nonaugmented controls and a commercially available scaffold. However, perhaps the most important finding of this study was that the control group demonstrated a similar outcome to augmented repairs.

Level of evidence: Basic Science Study; In Vivo Animal Model

Keywords: Animal model; enthesis healing; rotator cuff; scaffold; shoulder; tendon-bone; tissue engineering

All animal work was conducted in accordance with a Project License protocol accepted under the UK Home Office Animals (Scientific Procedures) Act 1986.

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Tendon-bone healing is an important factor affecting the outcome of rotator cuff repair.[6] Anatomic reattachment of the rotator cuff to its bone insertion is crucial, but tendon degeneration and poor bone quality at the enthesis compromise the quality, healing capacity, and durability of the performed

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repair.[14] To absorb the energy of loading between tendon and bone, the native enthesis comprises a natural gradation of 4 histologic zones (tendon, demineralized fibrocartilage, mineralized fibrocartilage, and bone).[18] After injury, this is replaced by a weak fibrovascular bridge with inferior biomechanical properties.[4,7,10] Osteopenia at the greater tuberosity often accompanies this change in structure, leading to a reduction in the pullout strength of suture anchors.[1,3,15,21]

Demineralized bone matrix (DBM) is an osteoinductive agent that consists of a collagen scaffold containing several growth factors. It has been demonstrated in vivo to regenerate a fibrocartilaginous enthesis capable of resisting physiologic forces but has not been investigated in a degenerative model of tendon-bone healing.[12,16,17] The purpose of this study was to assess the effect of DBM on regeneration of an enthesis after repair of a degenerative rotator cuff tear. We compared DBM with another commercially available augmentation product with clinically investigated profiles of activity (GraftJacket; Wright Medical Technology, Inc., Arlington, TN, USA). GraftJacket is obtained from donated human cadaveric dermal tissue processed to remove its cellular components while retaining its extracellular matrix. Its acellularity has the advantage of not causing a host inflammatory reaction, and it has been safely used in rats to enhance healing of a large acute rotator cuff tear.[8] The hypothesis was that augmentation with DBM will result in a higher bone mineral density in the recipient footprint and regenerate a morphologically superior enthesis characterized by greater fibrocartilage formation and improved collagen fiber organization in a rat model of chronic rotator cuff degeneration compared with acellular human dermal matrix after repair of a tendon tear.

Materials and methods

Study design

In this basic science study, 18 female Wistar rats underwent unilateral detachment of the supraspinatus tendon. Previously published data were used to calculate the number of animals (n = 6) required to generate a power of 0.8 with significance at the .05 level.[16] Three weeks later, tendon repair was carried out in animals randomized into 3 groups: group 1 (n = 6) received augmentation of the repair with cortical allogeneic DBM; group 2 (n = 6) received augmentation with nonmeshed, ultrathick acellular human dermal matrix (GraftJacket; average of 1.4 mm in thickness); and group 3 (n = 6) underwent direct tendon-bone repair without augmentation. One surgeon carried out all procedures using a standard technique. Animals were allowed to mobilize freely after surgery. Specimens were retrieved at 6 weeks postoperatively for histologic analysis and peripheral quantitative computed tomography (pQCT) to evaluate bone mineral density at the reattachment footprint of the tendon, reversal of degenerative changes within the tendon, and histologic remodeling of the implanted augmentation material.

DBM manufacture

DBM derived from cortical bone was manufactured according to Urist's protocol, with modifications.[20] The tibiae of skeletally mature female Wistar rats were harvested immediately after euthanasia; all soft tissues and periosteum were stripped from the bone surface. Bones measuring approximately 30 mm in length by 3 mm in width were demineralized in 0.6 N HCl at room temperature. Demineralization was confirmed by taking radiographs (300 seconds, 30 kV; Faxitron Corporation, Lincolnshire, IL, USA). This was followed by washing in phosphate-buffered saline until the pH was 7.4 ± 0.1. Samples were stored at −20°C for 2 hours and transferred to a lyophilizer (Edwards Girovac Ltd., Crawley, West Sussex, UK) for 3 days. Specimens were then sealed in individual plastic bags, sterilized by gamma irradiation at a dose of 25 kGy (Isotron Limited, Reading, UK), and stored at −20°C. Samples were rehydrated at the time of surgery in normal saline for 30 minutes before use.

Surgical technique

Two operations were performed on each animal: full-thickness supraspinatus tendon detachment and complete tendon reattachment. Anesthesia was induced and maintained using 2% isoflurane mixed with pure oxygen through a facemask for both procedures. The right shoulder was operated on in all cases. A 1.5-cm skin incision was made directly over the anterolateral border of the acromion. The deltoid was detached from the acromion and split caudally for 0.5 cm to identify the tendon of the supraspinatus. The supraspinatus tendon was completely detached from its bone insertion on the humeral head, marked with a 5-0 Prolene suture (Ethicon, Johnson & Johnson Medical Ltd., Berkshire, UK) at the musculotendinous junction, and allowed to retract medially. Deltoid muscle, superficial fascia, and skin were closed with 5-0 Vicryl suture (Ethicon). Animals were allowed unrestricted cage activity and received analgesia (subcutaneous buprenorphine) every 12 hours for 3 days. The second operation to reattach the tendon was undertaken 3 weeks after the first procedure. Before the skin incision was made, the DBM or GraftJacket was rehydrated for 30 minutes in sterile normal saline at the operating table.

A 2-cm skin incision was made in line with the supraspinatus muscle belly, ending anterior to the lateral end of the clavicle. This approach was perpendicular to the incision used for tendon detachment to make use of a virgin anatomic plane devoid of scar tissue. The muscle belly of the supraspinatus was identified and followed distally to reveal the tendon stump with the suture marker in the musculotendinous junction. Scar tissue between the tendon stump and its insertion was excised, and the tendon was grasped with a double-armed 5-0 Prolene suture using a modified Mason-Allen technique.[19] Despite traction on the tendon stump, it could not be directly brought back to the humeral head in any of the cases. The bare tendon-bone insertion footprint was decorticated with a No. 11 surgical blade until bleeding was seen. A custom-made dental drill was used to drill a 1-mm hole from the neck of the humerus to the bone insertion of the detached supraspinatus.

The scaffold (DBM or GraftJacket) was cut into a strip 10 mm long and 3 mm wide. Each limb of the suture was passed through the scaffold to secure it in position. One suture limb was passed through the hole in the prepared tendon stump, and the other suture limb was passed through the hole on the neck of the humerus. The supraspinatus tendon–scaffold complex was attached to the insertion site, with the graft in contact with both the tendon stump proximally and decorticated bone surface distally (Fig. 1). In the control group, the sutures were inserted directly into the drill holes, leaving a 5-mm gap between the tendon and bone in all cases.